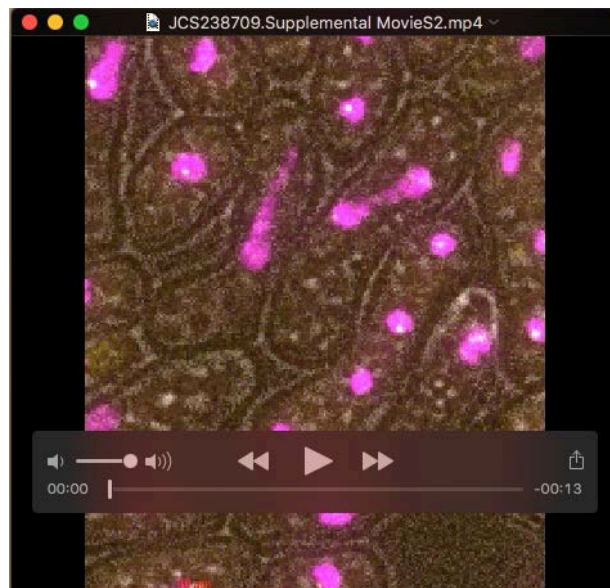


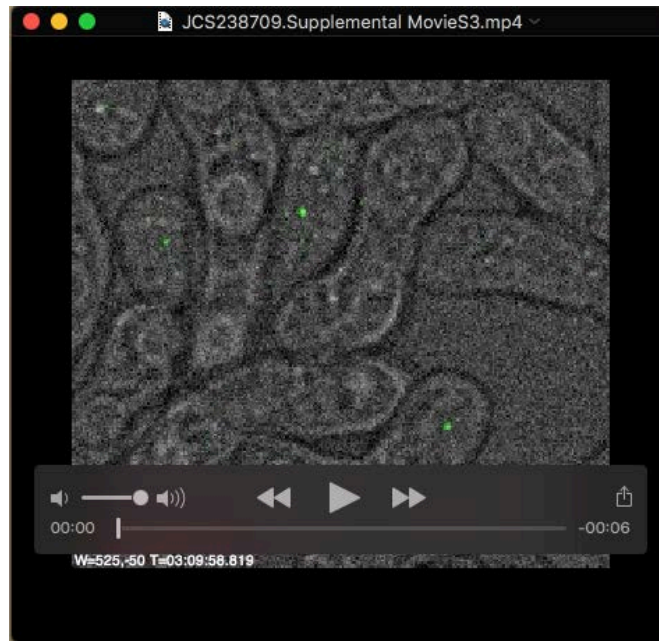
Figure S1: Western Blots of Rec8-GFP. This figure is related to Figure 4. Blots and total protein gels used in determining Rec8-GFP phosphorylation and abundances. Only one gel technical replicate for Experiment 1 is pictured although two technical replicates were completed and averaged. Experiment 5 is not pictured as there was only data for WT time points from -2, -1, and 0 available for analysis.



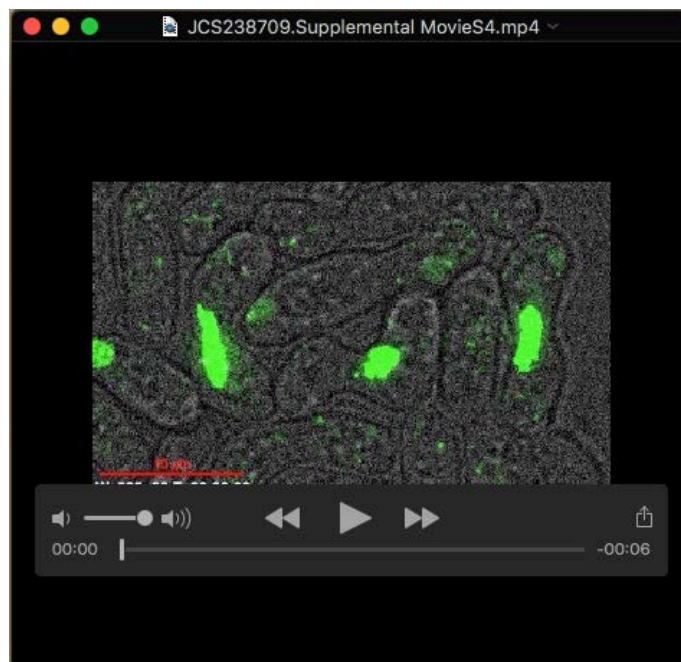
Movie 1: Live cell imaging of WT chromosome segregation. Representative live cell imaging of WT (5608x5787) heterozygote LacI-GFP and *lacO* at centromere I with H3-mRFP in meiosis. Yellow is LacI-GFP and magenta is H3-mRFP.



Movie 2: Live cell imaging of *quad*Δ chromosome segregation. Representative live cell imaging of *quad*Δ (6671x7117) heterozygote LacI-GFP and *lacO* at centromere I with H3-mRFP in meiosis. Yellow is LacI-GFP and magenta is H3-mRFP.



Movie 3: Live cell imaging of WT Rec8 cohesin. Representative live cell imaging of WT (6137x6138) with Rec8-GFP in meiosis. Green is Rec8-GFP.



Movie 4: Live cell imaging of *quad* Δ Rec8 cohesin. Representative live cell imaging of *quad* Δ (7428x7402) with Rec8-GFP in meiosis. Green is Rec8-GFP.



Movie 5: Live cell imaging of WT Rad21 cohesin. Representative live cell imaging of WT (7644x7645) with Rad21-GFP and H3-mRFP in meiosis. Yellow is Rad21-GFP and magenta is H3-mRFP



Movie 6: Live cell imaging of *quad*Δ Rad21 cohesin. Representative live cell imaging of *quad*Δ (7633x7634) with Rad21-GFP and H3-mRFP in meiosis. Yellow is Rad21-GFP and magenta is H3-mRFP

Table S1: Strains used in this study.

Strain	Genotype	Source
1251	<i>h+ ade6-M26 his4-239</i>	Gerry Smith
2057	<i>h- pat1-114 ade6-M216 can1-1</i>	Mastro 2014
3070	<i>h- Δrad3::ura4+ ura4-D18</i>	Lab Stock
3500	<i>h90 mat2-102 pat1-114 ade6-M210</i>	Mastro 2014
4561	<i>h+ Δrec12::ura4+ ura4-D18 his4-239 ade6-M26</i>	This Study
5207	<i>h- lys4-95 ade6-52</i>	Mastro 2014
5258	<i>h- Δkpa1::bleMX6 lys4-95 ade6-52</i>	This Study
5259	<i>h- Δrev3::hphMX6 lys4-95 ade6-52</i>	This Study
5262	<i>h- eso1Δη::kanMX6 lys4-95 ade6-52</i>	This Study
5263	<i>h+ Δrev3::hghMX6 his4-239 ade6-M26</i>	This Study
5268	<i>h- Δrec12::ura4+ ura4-D18 ade6-52 lys4-95</i>	This Study
5269	<i>h+ eso1Δη::kanMX6 his4-239 ade6-M26</i>	This Study
5401	<i>h+ Δrev1::ura4+ ura4-D18 his4-239 ade6-M26</i>	This Study
5466	<i>h- Δrev1::ura4+ ura4-D18 lys4-95 ade6-52</i>	This Study
5608	<i>h- hht1-mRFP:KanMX6 leu1-32 ura4-D18</i>	Mastro 2014
5787	<i>h+ hht1-mRFP:kanMX his7+::lacI-GFP lys1+::lacO leu1-32 ura4-D18</i>	Mastro 2014
6173	<i>h+ rec8-GFP-kan(YW) leu1-32 ade6-M210</i>	This Study
6174	<i>h- rec8-GFP-kan(YW) leu1-32 ade6-M210</i>	This Study
6332	<i>h90 mat2-102 pat1-114 rec8-GFP-kan(YW) ade6-M210</i>	Mastro 2014
6336	<i>h- pat1-114 rec8-GFP-kan(YW) ade6-M216</i>	Mastro 2014
6664	<i>h- his4-239 eso1Δη::kanMX6 Δkpa1::bleMX6 Δrev3::hphMX6 Δrev1::ura4+ ura-D18 ade6-</i>	This Study
6671	<i>h90 mat2-101 pat1-114 eso1η::kanMX6 Δkpa1::bleMX6 Δrev3::hphMX6 Δrev1::ura4+ ura-D18 leu1-32 ade6-M210</i>	This Study
6703	<i>h+ eso1Δη::kanMX6 Δkpa1::bleMX6 Δrev3::hphMX6 Δrev1::ura4+ ura-D18 his4-239 ade6-m26?</i>	This Study
6716	<i>h+ eso1Δη::kanMX6 Δkpa1::bleMX6 Δrev3::hphMX6 Δrev1::ura4+ ura-D18 lys4-95 ade6-52</i>	This Study
6717	<i>h- eso1Δη::kanMX6 Δkpa1::bleMX6 Δrev3::hphMX6 Δrev1::ura4+ ura-D18 lys4-95 ade6-52</i>	This Study
7117	<i>h- eso1Δη::kanMX6 Δkpa1::bleMX6 Δrev3::hphMX6 Δrev1::ura4+ his7+::lacI-GFP lys1+::lacO hht1-mRFP:natMX6 ura4-D18 leu1-32 ade6-</i>	This Study
7167	<i>h- pat1-114 eso1Δη::kanMX6 Δkpa1::bleMX6 Δrev3::hphMX6 Δrev1::ura4+ ura-D18 leu1-32 ade6-M216</i>	This Study
7168	<i>h+ eso1Δη::kanMX6 Δkpa1::bleMX6 Δrev3::hphMX6 Δrev1::ura4+ hht1-mRFP:natMX6 ura4-D18 leu1-32 ade6-</i>	This Study
7402	<i>h- pat1-114 rec8-GFP::kanMX6 eso1Δη::kanMX6 Δkpa1::bleMX6 Δrev3::hphMX6 Δrev1::ura4+ ura-D18 ade6-M216</i>	This Study
7501	<i>h90 mat2-102 pat1-114 eso1Δη::kanMX6 kpa1Δ::bleMX6 rec8-gfp:kanMX rev3::hphMX6 Δrev1::ura4+ ura-D18 ade6-M210</i>	This Study
7616	<i>h+ Δkpa1::bleMX6 his4-239 ade6-</i>	This Study

7633	<i>h- leu1 rad21-GFP[leu2] eso1Δη::kanMX6 Δkpa1::bleMX6 Δrev3::hphMX6 Δrev1::ura4+ hht1-mRFP:natMX6 ura4-D18 leu1-32 ade6(-)?</i>	This Study
7634	<i>h+ leu1 rad21-GFP[leu2] eso1Δη::kanMX6 Δkpa1::bleMX6 Δrev3::hphMX6 Δrev1::ura4+ hht1-mRFP:natMX6 ura4-D18 leu1-32 ade6(-)?</i>	This Study
7644	<i>h- leu1 rad21-GFP[leu2] hht1-mRFP:kanMX</i>	This Study
7645	<i>h+ leu1 rad21-GFP[leu2] hht1-mRFP:kanMX</i>	This Study
7685	<i>h- Δkpa1:bleMX6 lys4-95 ade6-</i>	This Study
7691	<i>h- eso1Δη::kanMX6 Δkpa1::bleMX6 Δrev3::hphMX6 Δrev1::ura4+ taz1-GFP::kanMX6 hht1-mRFP:KanMX6 ura4-D18 leu1-32 ade6(-)?</i>	This Study
7692	<i>h+ eso1Δη::kanMX6 Δkpa1::bleMX6 Δrev3::hphMX6 Δrev1::ura4+ taz1-GFP::kanMX6 hht1-mRFP:KanMX6 ura4-D18 leu1-32 ade6(-)?</i>	This Study

Table S2: Allele Sources for strains derived through crosses in this study.

Allele	Source
<i>Δrev3::hphMX6</i>	Derived from Matthew O'Connell's #2753
<i>eso1Δη::kanMX6</i>	Matthew O'Connell's #2751
<i>Δrev1::ura4+</i>	Derived from Thomas Kelly's AJC-F53
<i>Δkpa1:bleMX6</i>	Matthew O'Connell's #2752
<i>hht1-mRFP:KanMX6</i>	Derived from Julie Cooper's JCF5474
<i>his7+::lacI-GFP lys1+::lacO</i>	Derived from strain from M. Yanagida. Described in (Nabeshima et al., 1998)
<i>rec8-GFP-kan</i>	Derived from Y. Watanabe (PY204)
<i>Rad21-GFP[leu2]</i>	Derived from yeast genetic resource center japan FY10969
<i>Taz1-GFP::kanMX6</i>	Derived from Yasushi Hiraoka. Described in (Chikashige and Hiraoka, 2001)

Table S3: Biological replicate numbers used in western blotting.

Time Point	Rec8-GFP Phosphorylation		Rec8-GFP / Total Protein	
	WT	quadΔ	WT	quadΔ
-6	n/a	n/a	4	3
-5	n/a	n/a	4	4
-4	4	4	4	4
-3	4	4	4	4
-2	5	4	4	4
-1	5	4	4	4
0	5	4	4	4
1	3	3	3	3
2	n/a	n/a	2	2